Biochemical and Biophysical Research Communications 507 (2018) 437–442

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

AtMYB44 interacts with TOPLESS-RELATED corepressors to suppress *protein phosphatase 2C* gene transcription

Nguyen Hoai Nguyen, Jong-Joo Cheong*

Center for Food and Bioconvergence, Seoul National University, Seoul, 08826, Republic of Korea

ARTICLE INFO

Article history:
Received 2 November 2018
Accepted 10 November 2018
Available online 15 November 2018

Keywords:
AtMYB44
EAR motif
Histone deacetylase
Protein phosphatase 2C
Repressor
TOPLESS-RELATED corepressor

ABSTRACT

AtMYB44 has been described in diverse hormonal signaling processes including abscisic acid (ABA)-mediated tolerance to abiotic stress; however, its function as a transcription factor is controversial. AtMYB44 contains the amino acid sequence LSLSL, a putative ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR-ASSOCIATED AMPHIPHILIC REPRESSION (EAR) motif. In yeast two-hybrid assay, physical interaction between AtMYB44 and a TOPLESS-RELATED (TPR) corepressor was observed, but abolished by mutation of the EAR motif. We performed bimolecular fluorescence complementation assay to confirm their interaction *in planta*. Chromatin immunoprecipitation assay revealed binding of AtMYB44 to the promoter regions of clade A *protein phosphatase 2C* (*PP2C*) genes (e.g., *ABI1*, *ABI2*, and *HAI1*), implying putative targets. Levels of histone H3 acetylation around the promoter regions were markedly lower in *AtMYB44*-overexpressing (*35S:AtMYB44*) plants than in wild-type plants. These results suggest that AtMYB44 forms a complex with TPR corepressors and recruits histone deacetylase(s) to suppress *PP2C* gene transcription in a signal-independent manner.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

The Arabidopsis R2R3 transcription factor AtMYB44 has been described in diverse hormonal signaling processes, including abscisic acid (ABA)-mediated tolerance to abiotic stress [1–3] and seed germination [4], ethylene-modulating insect defense [5,6], and disease resistance mediated by salicylic acid and jasmonic acid [7,8].

AtMYB44 appears to act as a repressor of gene transcription in ABA-mediated stress responses. In *AtMYB44-overexpression* (*35S:AtMYB44*) transgenic Arabidopsis, expression of a group of Clade A *PP2C* genes, including *ABI1*, *ABI2*, *AtPP2CA*, *HAB1*, and *HAB2*, was diminished under salt stress [1]. *PP2C* genes encode a group of protein phosphatases that counteract protein kinases, thereby inhibiting the ABA-mediated signaling process [9,10]. Meanwhile, transgenic Arabidopsis, soybean, and rice seedlings overexpressing the *AtMYB44* gene exhibit enhanced drought/salt-stress tolerance [1,11,12], presumably owing to the repression of *PP2C* gene transcription. Additionally, we recently observed that AtMYB44 binds to its own gene promoter to repress gene transcription [13].

PP2Cs and sucrose non-fermenting 1-related protein kinases (SnRKs) act as negative and positive regulators of ABA signaling processes, respectively [9,10]. PP2Cs physically interact with SnRK2s to form complexes, inactivating the kinases via dephosphorylation [14]. Under abiotic stress conditions, the soluble ABA receptors PYR/PYL/RCAR capture ABA and consequently bind to PP2Cs, inhibiting phosphatase activity [9,15]. Then, SnRK2s are released from the PP2C–SnRK2 complex and phosphorylate ABA-responsive element binding factors (AREB/ABFs), which activate the expression of ABA-responsive genes [16].

A series of independent studies has suggested that AtMYB44 physically interacts with the ABA receptors (PYL8 and PYL9) and blocks the interaction with PP2Cs [2,17,18]. In particular, Jaradat et al. [2] reported that AtMYB44 physically interacts with PYL8 and represses ABA signaling in response to drought and senescence. Additionally, several studies have described AtMYB44 as a phosphorylation-dependent positive regulator of ABA signaling [3,4]. In particular, Persak and Pitzschke [19] performed a dominant repression study under salt stress conditions and proposed that AtMYB44 functions as a transcriptional activator of tolerance-enhancing factors rather than as a repressor of tolerance-diminishing factors. In such cases, AtMYB44 acts as a negative regulator of ABA signaling and stress responses, in contrast to our previous observation of the positive role of AtMYB44 in ABA

* Corresponding author. Center for Food and Bioconvergence, Seoul National University, Seoul, 08826, Republic of Korea.
E-mail address: cheongjj@snu.ac.kr (J.-J. Cheong).

https://doi.org/10.1016/j.bbrc.2018.11.057
0006-291X/© 2018 Elsevier Inc. All rights reserved.

responses [1]. Moreover, Shim et al. [7] reported that AtMYB44 acted as a transcriptional activator of *WRKY70* by directly binding to the promoter in the defense response against a necrotrophic pathogen.

In this study, we investigated the role of AtMYB44 as a repressive transcription factor regulating the expression of clade A *PP2Cs*, including *ABI1*, *ABI2*, and *HAI1*. We observed that AtMYB44 physically interacts with TPR corepressors through the EAR motif (LxLxL, where conserved leucine residues are underlined) in the C-terminal catalytic domain. AtMYB44 forms a complex with TPR corepressors and represses the transcription of *PP2Cs* via the promotion of histone deacetylation at the gene locus. These results support the role of AtMYB44 as a repressor of *PP2C* gene expression and a positive regulator of ABA responses.

2. Materials and methods

2.1. Plant materials and growth conditions

All mutants and transgenic Arabidopsis plants used in this work had the ecotype Columbia (Col-0) background. The *atmyb44* mutant (SALK-039074) and homozygous T5 seeds of *35S:AtMYB44* #21 and 35S:*AtMYB44-GFP* #28-3 [1] were used. The seeds were surface-sterilized and sown on half-strength Murashige and Skoog (1/2 × MS) medium (2% sucrose). After storage at 4 °C for 3 days for stratification, seeds were geminated and grown in a growth chamber with a 16/8-h light/dark cycle at 23 ± 1 °C and ~100 μmol photons m^{-2} s^{-1}.

2.2. Yeast two-hybrid assay

Full-length cDNAs of *AtMYB44* (EST 119B8) obtained from The Arabidopsis Information Resource (TAIR) and of *TPR1* (*AT1G80490.2*) and *TPR3* (*AT5G27030.1*) obtained from RIKEN BioResource Center (Ibaraki, Japan) were amplified by PCR using specific primers (Table S1). The DNA fragments were cloned separately into *pGBKT7*, resulting in fusion of the Gal4 DNA-binding domain (BD) to the N-terminus of the tested protein, and into *pGADT7*, to fuse the Gal4 activation domain (AD) to the N-terminus of the tested protein (Takara Bio USA, Inc., Mountain View, CA, USA). These constructs were co-transformed into the yeast strain PBN204. Then, the transformed cells were separately dropped on master plate synthetic defined (SD) medium without leucine and tryptophan (SD-LW) and selection medium SD medium without leucine, tryptophan, and adenosine (SD-LWA). Positive control yeast cells were transformed with *pGBKT7-53* (the Gal4 DNA-binding domain fused with murine p53) and *pGADT7-T* (the Gal4 activation domain fused with SV40 large T-antigen).

Mutations in the EAR motif of AtMYB44 (*AtMYB44*-mEAR; L194A and L196A) were generated using the Muta-Direct™ Site Directed Mutagenesis kit (iNtRON Biotechnology, Sungnam, Korea) with specific primers (Table S1).

2.3. Bimolecular fluorescence complementation (BiFC) assay

The full length cDNAs of *AtMYB44*, *TPR1*, and *TPR3* were cloned respectively into the *pDONR^{TM}201* vector (Invitrogen, Boston, USA), and subcloned into *pBA3130*, *pBA3132*, *pBA3134*, and *pBA3136* vectors [20] using Gateway™ LR Clonase™ II Enzyme mix (Invitrogen, Boston, USA). The constructs were transformed into *Agrobacterium tumefaciens* (GV3101) and used for co-infiltration into leaves of 6–8-week-old tobacco plants (*Nicotiana benthamiana*). The *Agrobacterium* cells were cultured overnight, harvested, and resuspended in MM buffer (10 mM $MgCl_2$ and 10 mM MES; pH 5.5) supplemented with 100 μM acetosyringone and incubated at 28 °C for 1 h in a shaking incubator. The *Agrobacterium*-harboring p19 protein construct was also included in each experiment. The mixed cells were infiltrated into tobacco leaves with a needleless syringe. The plants were grown in a growth chamber for 2–3 additional days, and YFP fluorescence was observed in the leaf epidermal cells as described by Maple et al. [21] under a confocal microscope (Leica TCS SP8 X; Wetzlar, Germany).

2.4. Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed using the EpiQuik™ Plant ChIP Kit (EpiGentek, Farmingdale, NY, USA) according to the manufacturer's instructions. The anti-acetyl-histone H3 (H3ac; Merck Millipore 06-599, Burlington, USA) antibodies (2 μg per reaction) was used in the immunoprecipitation step. In the case of anti-GFP (Abcam ab290),

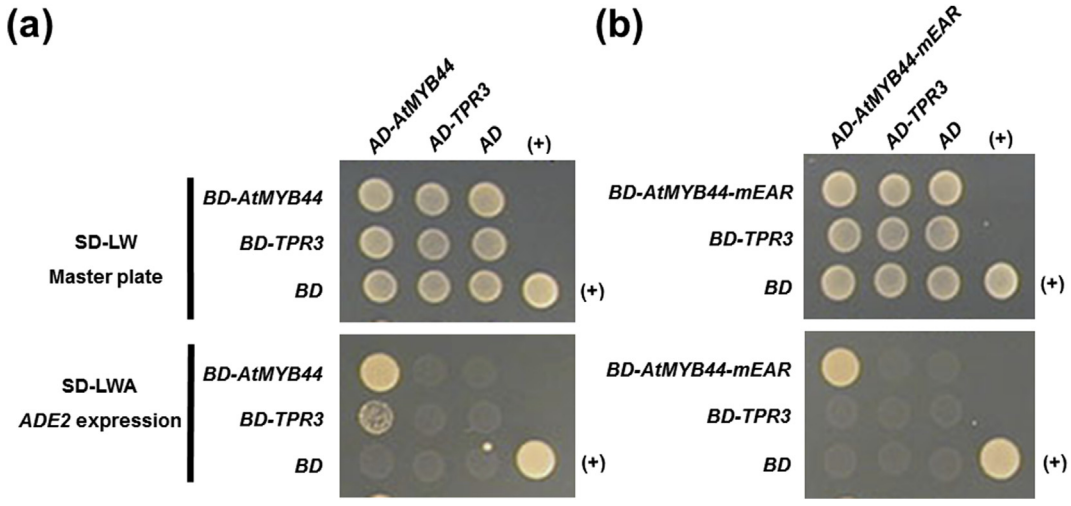

Fig. 1. Interaction of AtMYB44 and TPR3 corepressor in the yeast two-hybrid analysis.
The full-length coding DNA sequences of indicated genes were cloned into *pGBKT7-53* (for DNA-binding domain, BD) and *pGADT7* (for activation domain, AD), respectively. The constructs were co-transformed into the yeast strain PBN204, and the transformed cells were separately dropped onto master plate SD-LW and selection medium SD-LWA. Positive control yeast cells indicated by (+) were transformed with *pGBKT7-53* (the Gal4 DNA-binding domain fused with murine p53) and *pGADT7-T* (the Gal4 activation domain fused with SV40 large T-antigen).

5 μg was used per reaction, and 1 μg per reaction of normal rabbit IgG (Merck Millipore 12-370) was used as the negative control. The purified DNA samples were used for quantitative polymerase chain reaction (qPCR) as described previously [22] using specific primers (Table S1). For the AtMYB44–GFP binding signal, 5% of the extracted chromatin was used as the "input sample" in the assays, and the percent input (IP/IN%) was calculated using formula $100 \times 2^{[(Ct-Input\ -\ 4.322)\ -\ Ct-ChIP]}$. Relative H3ac levels were calculated by normalizing the percent input of the tested DNA region to the percent input of internal control *ACTIN2*. For statistical analysis, three independent experiments of the ChIP-qPCR assays were performed, and significance was assessed using Duncan's test [23] at a 95% confidence level.

3. Results and discussion

3.1. AtMYB44 physically interacts with TPR corepressors in planta

AtMYB44 contains an LSLSL sequence at amino acid numbers 194–198, which is likely an EAR motif (LxLxL) (Fig. S1). The presence of an EAR motif implies that this transcription factor may work as a repressor in gene transcription [24]. In addition, many studies have suggested that the EAR motif mediates the direct interaction between transcription factors and other corepressor proteins, such as TOPLESS (TPL) and TPRs to exhibit specific gene transcription repression activity [24,25]. For example, Szemenyei et al. [26] showed that the auxin-response repressor protein IAA12/BDL physically interacted with TPL via the EAR motif. Causier et al. [27] performed a yeast two-hybrid screening assay and reported a group of proteins interacting with TPR corepressors (TPR1 and TPR3) including AtMYB44.

Here, we confirmed that AtMYB44 physically interacts with TPR3 in yeast cells. For yeast two-hybrid assays, the full-length coding DNA sequences of *AtMYB44* and *TPR3* were cloned into the *pGADT7* and *pGBKT7* vectors for fusion to the activation domain (AD) and binding domain (BD), respectively. These constructs were then cotransformed into yeast. Using selective SD medium without leucine, tryptophan, or adenosine, the combination of AD-AtMYB44 and BD-TPR3 showed mutual interaction (Fig. 1a). By contrast, BD-AtMYB44 did not bind to AD-TPR3 but rather to itself (AD-AtMYB44), forming homodimers, as observed previously by Ref. [3]. Fusion to BD appeared to cause unknown conformational changes in the AtMYB44 protein and/or to hinder the binding site of TPR3.

To determine whether the EAR motif drives the interaction with TPR3, the first and second leucine residues were replaced with alanine (L194A and L196A) to generate the AtMYB44-mEAR variant. AtMYB44-mEAR did not interact with TPR3 in yeast (Fig. 1b), implying that AtMYB44 forms a repressor complex with TPR proteins via the EAR motif. By contrast, binding between BD-AtMYB44-mEAR and AD-AtMYB44-mEAR to form homodimers was observed, indicating that homodimer formation by AtMYB44 is not accomplished via the EAR motif.

Persak and Pitzschke [19] introduced an artificial EAR motif into the C-terminal end of AtMYB44 by fusing a LDLDL peptide without mutation or deletion of the endogenous putative EAR motif. In contrast to the phenotypes observed in the *35S:AtMYB44* lines, overexpression of this fusion gene interfered with stress responses to salt and drought. They suggested that the tolerance-improving effect observed in *35S:AtMYB44* lines was most likely attributable to transcriptional activation of the genes involved in the prevention of excessive accumulation of reactive oxygen species. Thus, they proposed that AtMYB44-conferred stress resistance results from the induction of tolerance-enhancing factors rather than the repression of tolerance-diminishing factors. In relation to this conclusion, which is contrary to our observations of the repressive function of AtMYB44, we speculate that the additional EAR motif peptide at the C-terminus of AtMYB44 interferes with the interaction with other regulator(s) that collaborate to repress target gene transcription.

The interactions between AtMYB44 and TPR corepressors were confirmed *in planta* using bimolecular fluorescence complementation (BiFC) assay. *AtMYB44* and *TPR1* or *TPR3* cDNAs were fused with the C-terminal (YFP^C) or N-terminal (YFP^N) fragment of the yellow fluorescence protein (YFP) gene to generate YFP^C-*AtMYB44*, *TPR1*-YFP^N, and *TPR3*-YFP^N constructs. YFP signals were emitted in the nuclei of transgenic tobacco plants co-transformed with YPF^C-*AtMYB44* and *TPR1/3*-YFP^N constructs (Fig. 2). By contrast, plants

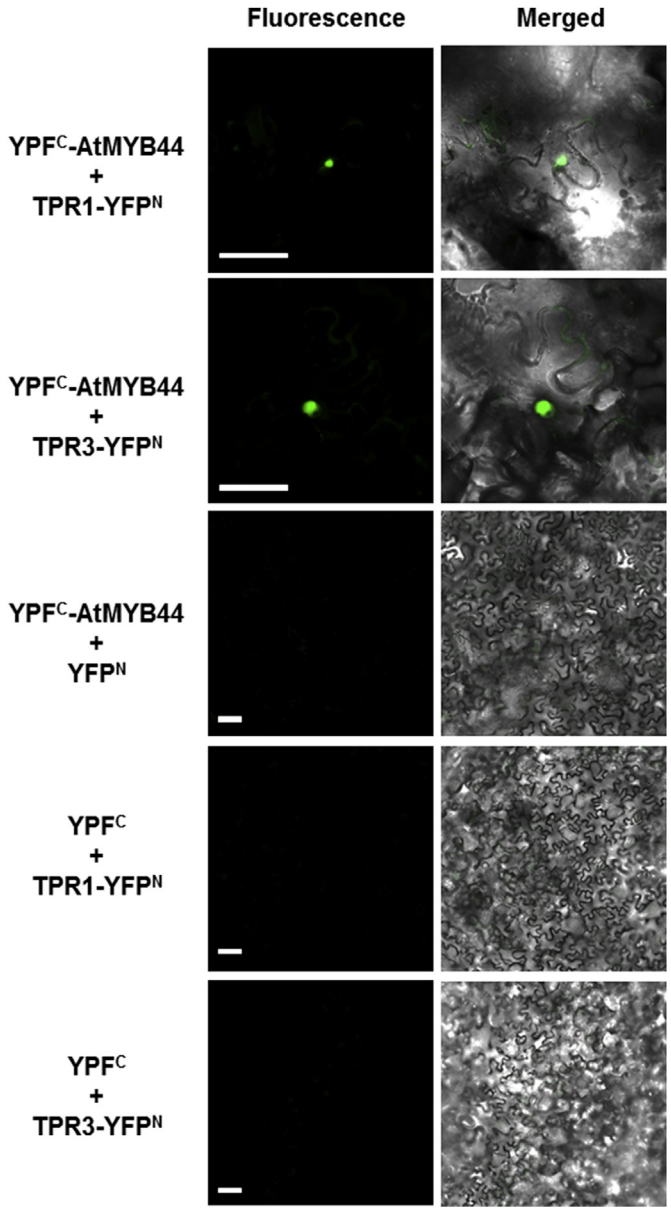

Fig. 2. Interaction of AtMYB44 and TPR corepressors in the BiFC analysis. *AtMYB44*, *TPR1*, and *TPR3* were fused with the C-terminal or N-terminal part of the *YFP* (YPF^C-*AtMYB44*, *TPR1*-YFP^N, and *TPR3*-YFP^N). *A. tumefaciens* (GV3101) containing the indicated constructs was co-infiltrated into tobacco (*N. benthamiana*) leaves. Fluorescence was detected in leaf epidermal cells under a confocal microscope (TCS SP8 X; Leica). Scale bar = 50 μm. Three independent experiments were performed, which yielded identical results.

transformed with an empty vector (YFP^C or YFP^N) and YFP^C-*AtMYB44*, *TPR1-YFP*^{N}, or *TPR3-YFP*^{N} did not exhibit YFP signals. This demonstrated that AtMYB44 forms a complex with either TPR1 or TPR3 proteins *in planta* independent of a hormone signal or external stimulus. When the YFP^C fused to the C-terminal side of AtMYB44 (*AtMYB44-YFP*^{C}) and co-transformed with *TPR1/3-YFP*^{N}, no YFP signal was observed (Fig. S2). We postulate that the YFP fragment at the C-terminus blocks the EAR motif, thereby inhibiting interaction between the two molecules. Li et al. [17] performed a BiFC assay and observed that AtMYB44 interacts with PYL9 (RCAR1), an ABA receptor, in Arabidopsis protoplast cells cotransformed with *AtMYB44-YFP*^{N} and *RCAR1-YFP*^{C} constructs. Thus, the interaction of AtMYB44 with TPR1/3 appears to differ from that with PYL9 in terms of physical mechanism.

3.2. AtMYB44 binds to PP2C promoters

In *AtMYB44-overexpression* (*35S:AtMYB44*) transgenic Arabidopsis, expression of a group of *PP2C* genes, including *ABI1*, *ABI2*, *AtPP2CA*, *HAB1*, and *HAB2*, was diminished under salt stress [1]. Thus, we suggested that clade A *PP2C* genes are putative targets of the repressive activity of AtMYB44.

AtMYB44 contains well-conserved R2R3 DNA-binding domains consisting of two repeats of 50–53-amino acids (Fig. S1). The tryptophan residues W9, W29, and W48 in the R2 domain and W80 and W99 in the R3 domain resided in the subgroup 22 MYB transcription factors conserved in the AtMYB44. Two lysine residues (K42 in R2 and K96 in R3) and one asparagine residue (N97 in R3) that recognize the specific DNA binding sequence AACnG [28], where n represents A, C, G, or T, were also well conserved. A protein binding microarray analysis confirmed AACnG as the consensus nucleotide sequence for the specific binding of AtMYB44 [29].

A number of the AtMYB44-binding sequences AACnG exists in the *PP2C* promoter (~1.5 kb) and gene-body regions, including three in the *ABI1* promoter, four in *ABI2*, and three in *HAI1* (Fig. 3a). Specific primer sets were designed against the nucleotide sequences of several clade A *PP2C* genes, including *ABI1*, *ABI2*, and *HAI1* (Fig. 3a and Table S1).

ChIP-qPCR assays with *35S: AtMYB44–GFP* plants [1] using a green fluorescent protein (GFP) antibody revealed that AtMYB44–GFP fusion proteins, at basal levels, bind directly to promoters, including the transcription start site proximal regions of the *ABI1*, *ABI2*, and *HAI1* genes (Fig. 3b).

3.3. AtMYB44 promotes histone deacetylation at PP2C loci

Studies have demonstrated that TPR repressors induce histone deacetylation to suppress target gene expression [24]. Histone deacetylation is known to compact targeted chromatin regions, leading to the repression of related genes [30]. For instance, TPL recruits histone deacetylases, such as HDA6 or HDA19, to form a repressor complex, which is involved in various signaling pathways [31–33]. Zhu et al. [34] also reported that TPR1 is associated with HDA19 and a disease resistance R protein (SNC1) to activate immune responses via the repression of negative regulators. Ryu et al. [35] performed a BiFC assay and observed that the transcription

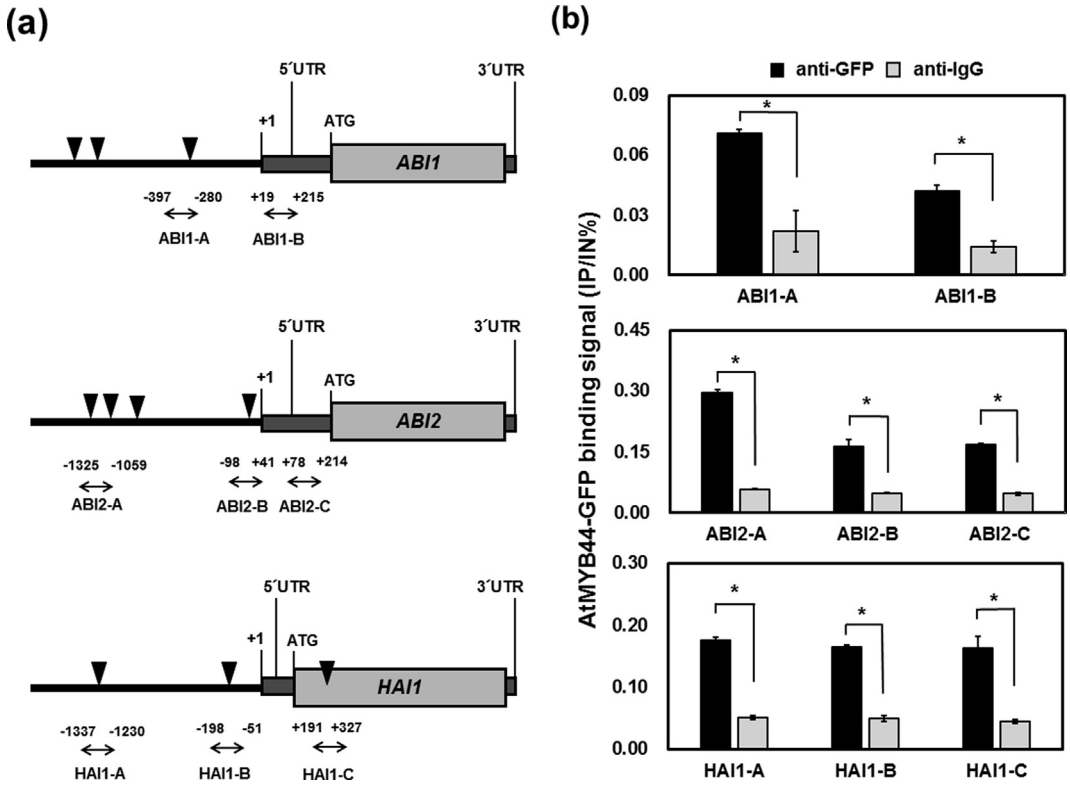

Fig. 3. Binding of AtMYB44 onto the *PP2C* loci.
(a) Schematic diagram of the *PP2C* loci. 5′UTR and 3′UTR represent the 5′- and 3′-untranslated regions. (+1) indicates the transcription start site (TSS), and ATG is the translation start codon. The triangles (▼) indicate the conserved AtMYB44-binding sites (AACnG). Specific primers (Table S1) were designed to target the indicated regions (A, B, and C) of each gene, as depicted in the diagram, and used for the ChIP-qPCR assays. (b) Binding of AtMYB44-GFP fusion proteins onto the *PP2C* loci. Two-week-old *AtMYB44-GFP* transgenic plants [1] grown on 1/2 × MS agar medium (2% sucrose) were used for the ChIP assays using antibodies against GFP. qPCR was performed using specific primers for *ABI1*, *ABI2*, and *HAI1* (Fig. 3a and Table S1). IgG was included as a negative control. The AtMYB44–GFP binding signal was calculated as Percent Input (IP/IN%). Data are reported as the means ± standard error of values obtained from three independent experiments performed in triplicate. Columns marked with an asterisk (*) differ significantly ($P < 0.05$).

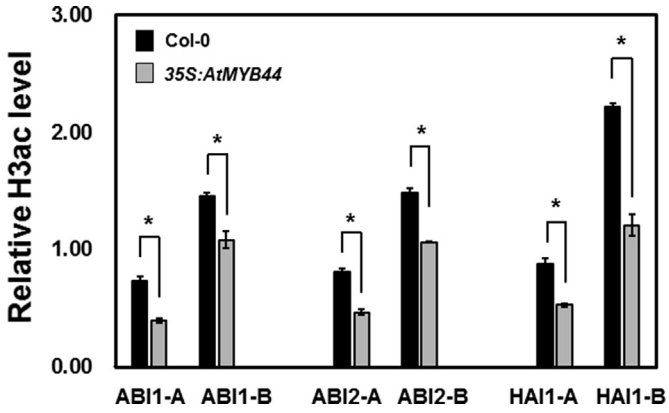

Fig. 4. Histone acetylation at the *PP2C* loci in *35S:AtMYB44* plants.
Two-week-old wild type (Col-0) and *35S:AtMYB44* plants [1] grown on 1/2 × MS agar medium (2% sucrose) were used for the ChIP assays. ChIP assays were conducted using antibodies against H3ac. qPCR was performed using specific primers indicated (Table S1). The ChIP-qPCR signal was normalized to the input DNA and an internal control (*ACTIN2*). Data are reported as the means ± standard error of values obtained from three independent experiments performed in triplicate. Columns marked with an asterisk (*) differ significantly ($P < 0.05$).

factor BES1 forms a repressor complex with TPL and HDA19. The authors noted that it remains unclear whether TPL-HDA19 interaction is direct or facilitated by adapter proteins.

We performed ChIP-qPCR assays to examine the status of histone acetylation at the *ABI1*, *ABI2*, and *HAI1* loci in wild-type and *35S:AtMYB44* transgenic plants. The levels of histone H3 acetylation (H3ac) at TSS proximal regions (regions B) of the *PP2Cs* were markedly lower in *35S:AtMYB44* plants than in wild-type plants (Fig. 4). Overall, these results indicate that the AtMYB44–TPR1/3 complex targets *PP2C* genes and promotes histone deacetylation at these loci to suppress gene transcription, supporting the role of AtMYB44 as a repressor of *PP2C* gene expression and a positive regulator of ABA responses.

Conflicts of interest

We have no conflict of interest to declare.

Acknowledgments

We would like to thank Prof. Choonkyun Jung (Seoul National University, Korea) and Dr. Phu-Tri Tran (Seoul National University, Korea) for providing vectors and microbial strains, respectively. This work was supported by the National Research Foundation of Korea (grant number 2016R1A2B4012248).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.11.057.

References

[1] C. Jung, J.S. Seo, S.W. Han, Y.J. Koo, C.H. Kim, S.I. Song, B.H. Nahm, Y.D. Choi, J.-J. Cheong, Overexpression of *AtMYB44* enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis, Plant Physiol. 146 (2008) 623–635.
[2] M.R. Jaradat, J.A. Feurtado, D. Huang, Y. Lu, A.J. Cutler, Multiple roles of the transcription factor AtMYBR1/AtMYB44 in ABA signaling, stress responses, and leaf senescence, BMC Plant Biol. 13 (2013) 192, https://doi.org/10.1186/1471-2229-13-192.
[3] H. Persak, A. Pitzschke, Tight interconnection and multi-level control of Arabidopsis MYB44 in MAPK cascade signaling, PloS One 8 (2013) e57547, https://doi.org/10.1371/journal.pone.0057547.
[4] X.C. Nguyen, M.H.T. Hoang, H.S. Kim, K. Lee, X.-M. Liu, S.H. Kim, S. Bahk, H.C. Park, W.S. Chung, Phosphorylation of the transcriptional regulator MYB44 by mitogen activated protein kinase regulates *Arabidopsis* seed germination, Biochem. Biophys. Res. Commun. 423 (2012) 703–708.
[5] R. Liu, L. Chen, Z. Jia, B. Lü, H. Shi, W. Shao, H. Dong, Transcription factor AtMYB44 regulates induced expression of the *ETHYLENE INSENSITIVE2* gene in *Arabidopsis* responding to a harpin protein, Mol. Plant Microbe Interact. 24 (2011) 377–389.
[6] B.B. Lü, X.-J. Li, W.-W. Sun, L. Li, R. Gao, Q. Zhu, S.-M. Tian, M.-Q. Fu, H.-L. Yu, X.-M. Tang, C.-L. Zhang, H.-S. Dong, AtMYB44 regulates resistance to the green peach aphid and diamondback moth by activating *EIN2*-affected defences in *Arabidopsis*, Plant Biol. 15 (2013) 841–850.
[7] J.S. Shim, C. Jung, S. Lee, K. Min, Y.-W. Lee, Y. Choi, J.S. Lee, J.T. Song, J.-K. Kim, Y.D. Choi, *AtMYB44* regulates *WRKY70* expression and modulates antagonistic interaction between salicylic acid and jasmonic acid signaling, Plant J. 73 (2013) 483–495.
[8] B. Zou, Z. Jia, S. Tian, X. Wang, Z. Gou, B. Lü, H. Dong, AtMYB44 positively modulates disease resistance to *Pseudomonas syringae* through the salicylic acid signalling pathway in *Arabidopsis*, Funct. Plant Biol. 40 (2013) 304–313.
[9] Y. Ma, I. Szostkiewicz, A. Korte, D. Moes, Y. Yang, A. Christmann, E. Grill, Regulators of PP2C phosphatase activity function as abscisic acid sensors, Science 324 (2009) 1064–1068.
[10] T. Umezawa, K. Nakashima, T. Miyakawa, T. Kuromori, M. Tanokura, K. Shinozaki, K. Yamaguchi-Shinozaki, Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport, Plant Cell Physiol. 51 (2010) 1821–1839.
[11] J.S. Seo, H.B. Sohn, K. Noh, C. Jung, J.H. An, C.M. Donovan, D.A. Somers, D.I. Kim, S.-C. Jeong, C.-G. Kim, H.M. Kim, S.-H. Lee, Y.D. Choi, T.W. Moon, C.H. Kim, J.-J. Cheong, Expression of the *Arabidopsis AtMYB44* gene confers drought/salt-stress tolerance in transgenic soybean, Mol. Breed. 29 (2012) 601–608.
[12] J. Joo, N.-I. Oh, N.H. Nguyen, Y.H. Lee, Y.-K. Kim, S.I. Song, J.-J. Cheong, Intergenic transformation of *AtMYB44* confers drought stress tolerance in rice seedlings, Appl. Biol. Chem. 60 (2017) 447–455.
[13] N.H. Nguyen, J.-J. Cheong, H2A.Z-containing nucleosomes are evicted to activate *AtMYB44* transcription in response to salt stress, Biochem. Biophys. Res. Commun. 499 (2018) 1039–1043.
[14] T. Umezawa, N. Sugiyama, M. Mizoguchi, S. Hayashi, F. Myouga, K. Yamaguchi-Shinozaki, Y. Ishihama, T. Hirayama, K. Shinozaki, Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in *Arabidopsis*, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 17588–17593.
[15] S.-Y. Park, P. Fung, N. Nishimura, D.R. Jensen, H. Fujii, Y. Zhao, et al., Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins, Science 324 (2009) 1068–1071.
[16] Y. Fujita, T. Yoshida, K. Yamaguchi-Shinozaki, Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants, Physiol. Plantarum 147 (2013) 15–27.
[17] D. Li, Y. Li, L. Zhang, X. Wang, Z. Zhao, Z. Tao, J. Wang, J. Wang, M. Lin, X. Li, Y. Yang, *Arabidopsis* ABA receptor RCAR1/PYL9 interacts with an R2R3-type MYB transcription factor, AtMYB44, Int. J. Mol. Sci. 15 (2014) 8473–8490.
[18] L. Xing, Y. Zhao, J. Gao, C. Xiang, J.K. Zhu, The ABA receptor PYL9 together with PYL8 plays an important role in regulating lateral root growth, Sci. Rep. 6 (2016) 27177, https://doi.org/10.1038/srep27177.
[19] H. Persak, A. Pitzschke, Dominant repression by *Arabidopsis* transcription factor MYB44 causes oxidative damage and hypersensitivity to abiotic stress, Int. J. Mol. Sci. 15 (2014) 2517–2537.
[20] J.S. Seo, H.-X. Sun, B.S. Park, C.-H. Huang, S.-D. Yeh, C. Jung, N.-H. Chua, ELF18-INDUCED LONG-NONCODING RNA associates with mediator to enhance expression of innate immune response genes in Arabidopsis, Plant Cell 29 (2017) 1024–1038.
[21] J. Maple, C. Aldridge, S.G. Møller, Plastid division is mediated by combinatorial assembly of plastid division proteins, Plant J. 43 (2005) 811–823.
[22] N.H. Nguyen, J.-J. Cheong, The *AtMYB44* promoter is accessible to signals that induce different chromatin modifications for gene transcription, Plant Physiol. Biochem. 130 (2018) 14–19.
[23] D.B. Duncan, Multiple range and multiple F tests, Biometrics 11 (1955) 1–42.
[24] S. Kagale, K. Rozwadowski, EAR motif-mediated transcriptional repression in plants: an underlying mechanism for epigenetic regulation of gene expression, Epigenetics 6 (2011) 141–146.
[25] M. Ikeda, M. Ohme-Takagi, A novel group of transcriptional repressors in Arabidopsis, Plant Cell Physiol. 50 (2009) 970–975.
[26] H. Szemenyei, M. Hannon, J.A. Long, TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis, Science 319 (2008) 1384–1386.
[27] B. Causier, M. Ashworth, W. Guo, B. Davies, The TOPLESS interactome: a framework for gene repression in Arabidopsis, Plant Physiol. 158 (2012) 423–438.
[28] K. Ogata, S. Morikawa, H. Nakamura, A. Sekikawa, T. Inoue, H. Kanai, A. Sarai, S. Ishii, Y. Nishimura, Solution structure of a specific DNA complex of the Myb DNA-binding domain with cooperative recognition helices, Cell 79 (1994) 639–648.
[29] C. Jung, Y.K. Kim, N.I. Oh, J.S. Shim, J.S. Seo, Y.D. Choi, B.H. Nahm, J.-J. Cheong, Quadruple 9-mer-based protein binding microarray analysis confirms AACnG as the consensus nucleotide sequence sufficient for the specific binding of AtMYB44, Mol. Cell. 34 (2012) 531–537.
[30] M.J. Pazin, J.T. Kadonaga, What's up and down with histone deacetylation and

transcription? Cell 89 (1997) 325–328.
[31] J.A. Long, C. Ohno, Z.R. Smith, E.M. Meyerowitz, TOPLESS regulates apical embryonic fate in *Arabidopsis*, Science 312 (2006) 1520–1523.
[32] N.T. Krogan, K. Hogan, J.A. Long, APETALA2 negatively regulates multiple floral organ identity genes in *Arabidopsis* by recruiting the co-repressor TOPLESS and the histone deacetylase HDA19, Development 139 (2012) 4180–4190.
[33] E. Oh, J.-Y. Zhu, H. Ryu, I. Hwang, Z.-Y. Wang, TOPLESS mediates brassinosteroid-induced transcriptional repression through interaction with BZR1, Nat. Commun. 5 (2014) 4140, https://doi.org/10.1038/ncomms5140.
[34] Z. Zhu, F. Xu, Y. Zhang, Y.T. Cheng, M. Wiermer, X. Li, Y. Zhang, Arabidopsis resistance protein SNC1 activates immune responses through association with a transcriptional corepressor, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 13960–13965.
[35] H. Ryu, H. Cho, W. Bae, I. Hwang, Control of early seedling development by BES1/TPL/HDA19-mediated epigenetic regulation of *ABI3*, Nat. Commun. 5 (2014) 4318, https://doi.org/10.1038/ncomms5138.